REPLACEMENT SHEET

OIPE IAP62 MAR 13 2006 PATENT & TRADEMARK OFFICE

Fig. 1

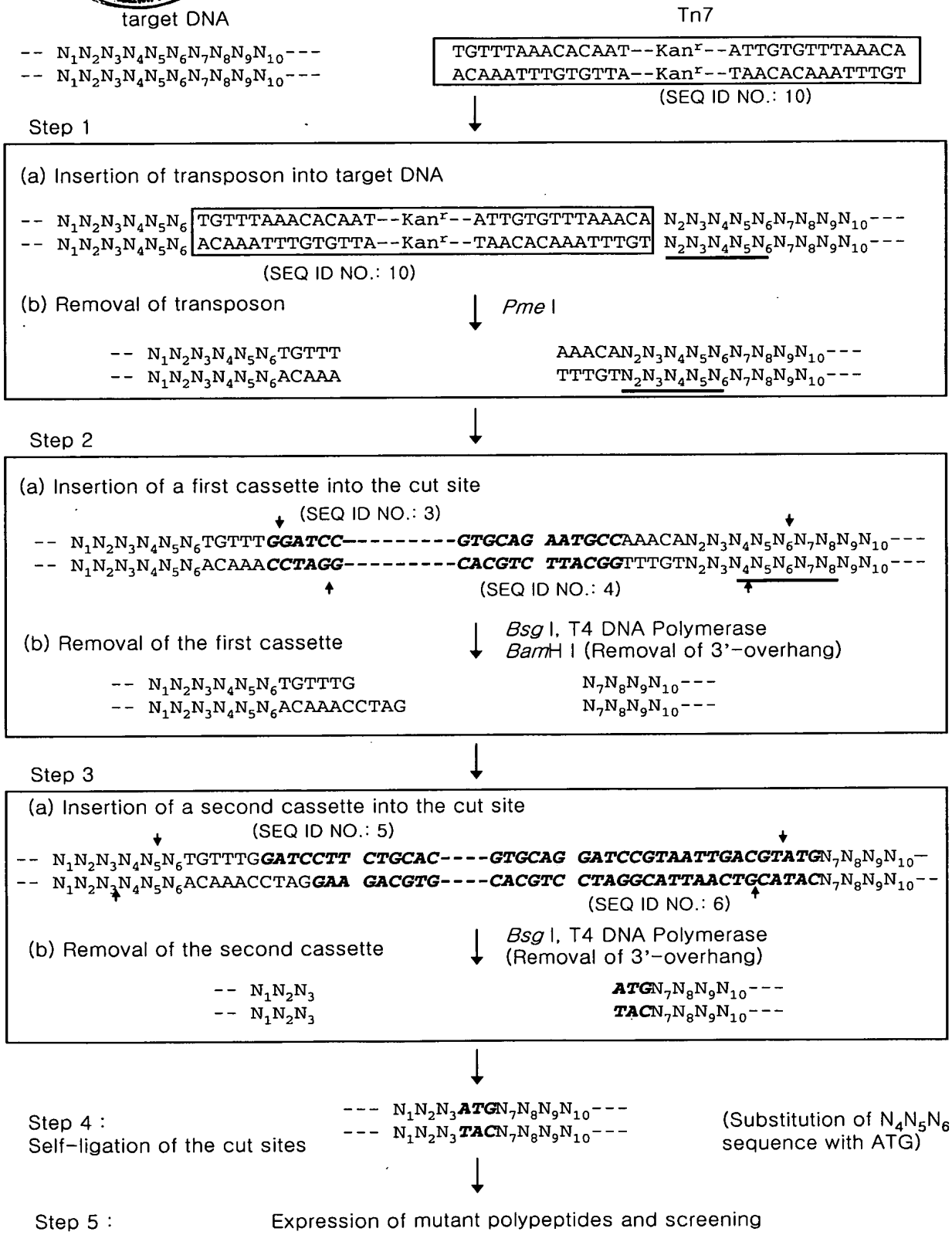

Fig. 2

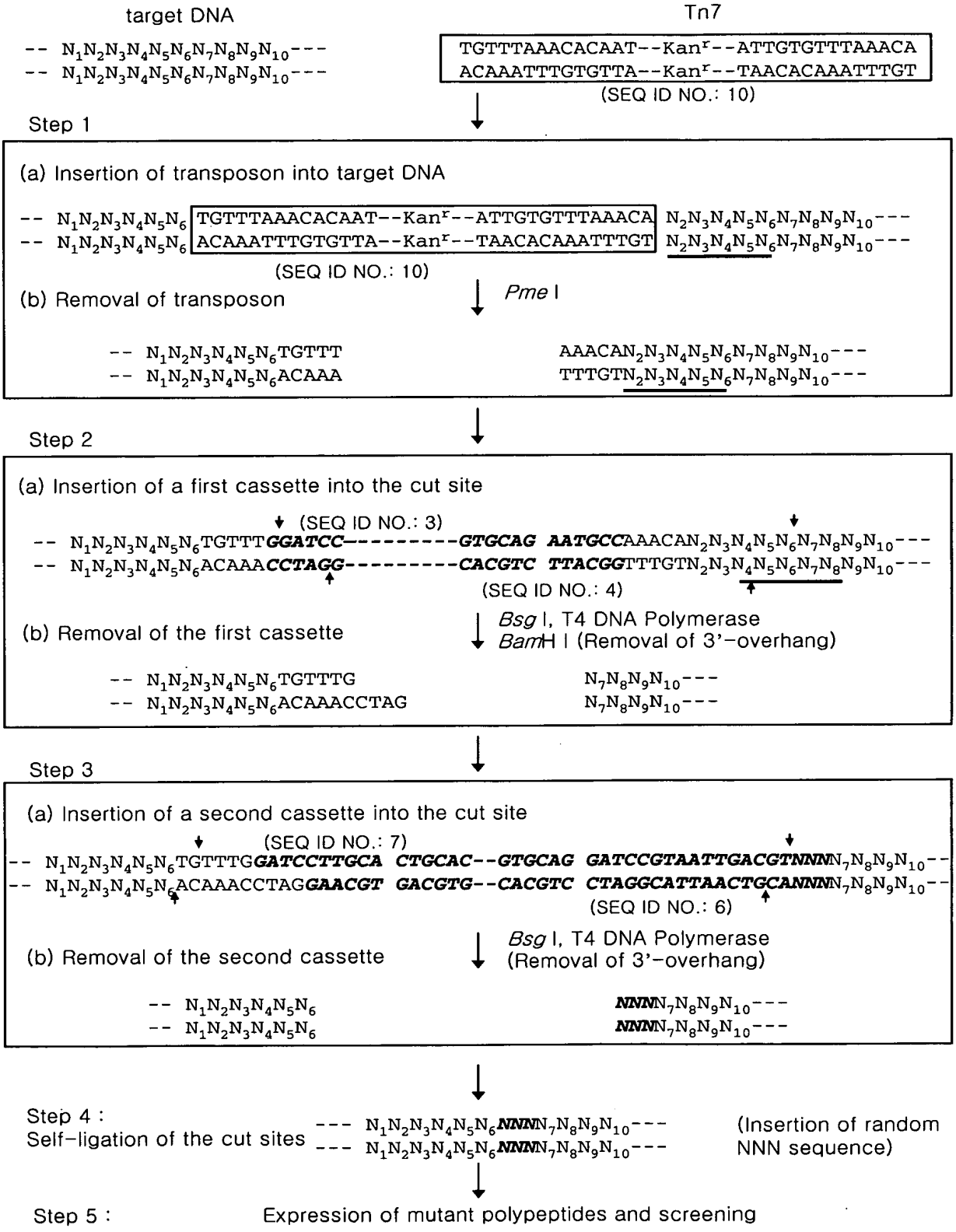
target DNA
Tn7
-- N1N2N3N4N5N6N7N8N9N10---
-- N1N2N3N4N5N6N7N8N9N10---
TGTTTAAACACAAT--Kanr--ATTGTGTTTAAACA
ACAAATTTGTGTTA--Kanr--TAACACAAATTTGT
(SEQ ID NO.: 10)
Step 1
(a) Insertion of transposon into target DNA
(SEQ ID NO.: 10)
(b) Removal of transposon
Pme I
-- N1N2N3N4N5N6TGTTT
-- N1N2N3N4N5N6ACAAA
AAACAN2N3N4N5N6N7N8N9N10---
TTTGTN2N3N4N5N6N7N8N9N10---
Step 2
(a) Insertion of a first cassette into the cut site
(SEQ ID NO.: 3)
(SEQ ID NO.: 4)
(b) Removal of the first cassette
Bsg I, T4 DNA Polymerase
BamH I (Removal of 3'-overhang)
-- N1N2N3N4N5N6TGTTTG
-- N1N2N3N4N5N6ACAAACCTAG
N7N8N9N10---
N7N8N9N10---
Step 3
(a) Insertion of a second cassette into the cut site
(SEQ ID NO.: 7)
(SEQ ID NO.: 6)
(b) Removal of the second cassette
Bsg I, T4 DNA Polymerase
(Removal of 3'-overhang)
-- N1N2N3N4N5N6
-- N1N2N3N4N5N6
NNNN7N8N9N10---
NNNN7N8N9N10---
Step 4 :
Self-ligation of the cut sites
--- N1N2N3N4N5N6NNNN7N8N9N10---
--- N1N2N3N4N5N6NNNN7N8N9N10---
(Insertion of random NNN sequence)
Step 5 :
Expression of mutant polypeptides and screening

Fig. 3

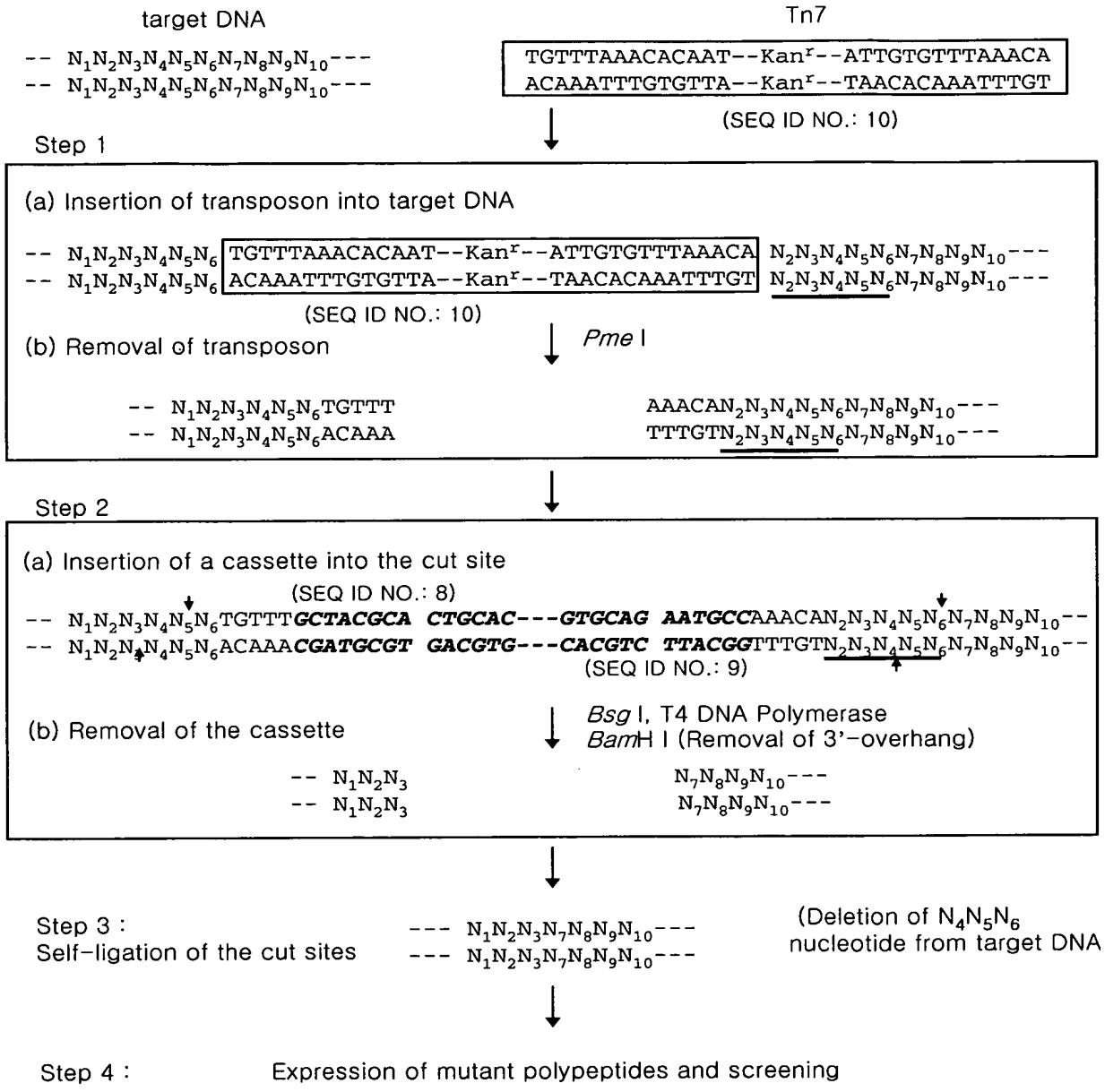
target DNA
-- $N_1N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$---
-- $N_1N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$---
Tn7
TGTTTAAACACAAT--Kan^r--ATTGTGTTTAAACA
ACAAATTTGTGTTA--Kan^r--TAACACAAATTTGT
(SEQ ID NO.: 10)
Step 1
(a) Insertion of transposon into target DNA
-- $N_1N_2N_3N_4N_5N_6$ TGTTTAAACACAAT--Kan^r--ATTGTGTTTAAACA $N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$---
-- $N_1N_2N_3N_4N_5N_6$ ACAAATTTGTGTTA--Kan^r--TAACACAAATTTGT $N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$---
(SEQ ID NO.: 10)
(b) Removal of transposon
Pme I
-- $N_1N_2N_3N_4N_5N_6$TGTTT
-- $N_1N_2N_3N_4N_5N_6$ACAAA
AAACA$N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$---
TTTGT$N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$---
Step 2
(a) Insertion of a cassette into the cut site
(SEQ ID NO.: 8)
-- $N_1N_2N_3N_4N_5N_6$TGTTT***GCTACGCA CTGCAC---GTGCAG AATGCC***AAACA$N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$--
-- $N_1N_2N_3N_4N_5N_6$ACAAA***CGATGCGT GACGTG---CACGTC TTACGG***TTTGT$N_2N_3N_4N_5N_6N_7N_8N_9N_{10}$--
(SEQ ID NO.: 9)
(b) Removal of the cassette
Bsg I, T4 DNA Polymerase
*Bam*H I (Removal of 3'-overhang)
-- $N_1N_2N_3$
-- $N_1N_2N_3$
$N_7N_8N_9N_{10}$---
$N_7N_8N_9N_{10}$---
Step 3 :
Self-ligation of the cut sites
--- $N_1N_2N_3N_7N_8N_9N_{10}$---
--- $N_1N_2N_3N_7N_8N_9N_{10}$---
(Deletion of $N_4N_5N_6$ nucleotide from target DNA
Step 4 :
Expression of mutant polypeptides and screening

Fig. 4

AlwI	GGATC(N)$_4$^ CCTAG(N)$_5$^	BsmAI	GTCTC(N)$_1$^ CAGAG(N)$_5$^	HphI	GGTGA(N)$_8$^ CCACT(N)$_7$^
BbsI	GAAGAC(N)$_2$^ CTTCTG(N)$_6$^	BsmB1	CGTCTC(N)$_1$^ GCAGAG(N)$_5$^	MboII	GAAGA(N)$_8$^ CTTCT(N)$_7$^
BbvI	GCAGC(N)$_8$^ CGTCG(N)$_{12}$^	BsmF1	GGGAC(N)$_{10}$^ CCCTG(N)$_{14}$^	MlyI	GAGTC(N)$_5$^ CTCAG(N)$_5$^
BciVI	GTATCC(N)$_6$^ CATAGG(N)$_5$^	BspM1	ACCTGC(N)$_4$^ TGGACG(N)$_8$^	PleI	GAGTC(N)$_4$^ CTCAG(N)$_5$^
BmrI	ACTGGG(N)$_5$^ TGACCC(N)$_4$^	BsrDI	GCAATG(N)$_2$^ CGTTAC^(N)$_2$	SapI	GCTCTTC(N)$_1$^ CGAGAAG(N)$_4$^
BpmI	CTGGAG(N)$_{16}$^ GACCTC(N)$_{14}$^	BtsI	GCAGTG(N)$_2$^ CGTCAC^(N)$_2$	SfaN1	GCATC(N)$_5$^ CGTAG(N)$_9$^
BsaI	GGTCTC(N)$_1$^ CCAGAG(N)$_5$^	EarI	CTCTTC(N)$_1$^ GAGAAG(N)$_4$^	FokI	GGATG(N)$_9$^ CCTAC(N)$_{13}$^
BseRI	GAGGAG(N)$_{10}$^ CTCCTC(N)$_8$^	EciI	GGCGGA(N)$_{11}$^ CCGCCT(N)$_9$^	HgaI	GACGC(N)$_5$^ CTGCG(N)$_{10}$^
BsgI	GTGCAG(N)$_{16}$^ CACGTC(N)$_{14}$^				